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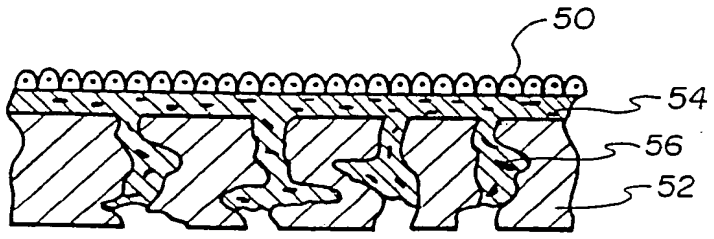
(54) Title: HOLLOW VISCUS PROSTHESIS AND METHOD OF IMPLANTATION



(57) Abstract

A prosthetic device capable of implantation in living tissue is disclosed which comprises a porous synthetic substrate (52) having opposing surfaces and collagenous material (54) in contact with the porous substrate. In a preferred embodiment, the substrate is comprised of synthetic material having irregular porosity. Fibroblasts (56) may also be in contact with the collagenous material (54) and the porous synthetic substrate (52). The invention may be used for repairing or replacing body tissues or viscera.

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HOLLOW VISCUS PROSTHESIS AND METHOD OF IMPLANTATION

BACKGROUND OF THE INVENTION

Field: This invention relates to products and techniques used in the repair or reconstruction of the walls of organs or other body parts which have been breached by accidental trauma or resected by surgical necessity. More particularly, this invention relates to implantable prostheses capable of being incorporated into the surrounding tissue for restoration of portions of walls of organs or replacement of entire organ walls when such organs or body parts are not susceptible to reconstruction by traditional techniques, such as grafting or end-to-end anastomosis.

State of the Art: There are numerous diseases or conditions for which replacement of a hollow viscus or partial reconstruction of an organ wall is indicated. The more common of these diseases or conditions are non-responding stricture of the esophagus; esophageal carcinoma, the incidence of which is reported as 1% of all malignant lesions and 4% of gastrointestinal malignancy; non-responding neurogenic bladder with large volume urinary retention, neurogenic bladder being present to some degree in probably 83% of diabetic patients; urinary tract obstruction which may involve ureter or urethra obstruction as the result of trauma, congenital defects or neoplasia; and neoplasia of the urinary bladder which comprises 2.5% of all cancer deaths.

There are many other diseases or conditions for which replacement or reconstruction of an organ or tissue area would be invaluable, but the technology for doing so has been lacking. Examples of these are various diseases, such as cancer or idiopathic mucosal ulcerative colitis, which frequently result in much pain, discomfort, and inconvenience due to the new stomal opening and collecting equipment which must be worn. There are also circumstances under which, for example, the trachea must be resected, and the removal of more than 8 centimeters of

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trachea precludes an end-to-end anastomotic repair. At present, there is no consistent tracheal substitute.

The aforementioned diseases and conditions have often been treated by surgical substitution of another hollow viscus for the diseased one, or by substitution of tissue from other organs, as in graft reconstructions. For example, the esophagus may be replaced by a substituted portion of the colon, or more commonly by esophagogastrectomy. The lower urinary tract may be treated by partial to total cystectomy with ureteroileostomy. Additionally, the use of a reservoir ileostomy and mucosal proctectomy with ileoanal anastomosis has been utilized following coelectomy, although more recent procedures using mechanical stomal occluding devices have been advanced.

All of these procedures require prolonged surgical manipulation, have a fair number of post-operative complications, and require, in some cases, an external collecting device. In addition, there are some obvious aesthetic effects which are unsatisfactory in some of these procedures.

In addition to the use of donor tissues or organ replacements to treat these conditions, polymers and metals, alone or in combination, have been used for esophageal or lower urinary tract substitution. Many materials have been suggested for use as prostheses for tracheal, esophageal, and urinary tract replacements. For example, E. F. Bergman, in "The Experimental Replacement of Portions of the Esophagus by a Plastic Tube," 135 Annals of Surgery, March 1952, pp. 337-43, suggested the use of methacrylate or polyethylene tubing as a replacement following esophageal surgery. See also, Battersby, et al., "Esophageal Replacement with Plastic Tubes," A.M.A. Archives of Surgery, 1954, pp. 400-09 (disclosing laminated layers of polyethylene and Nylon); Barnes, et al., "Replacement of Portion of Canine Esophagus with Composite Prosthesis and Greater Omentum," 64 Jr. of Thoracic and Cardiovascular Surgery, December, 1972, pp.

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892-96 (disclosing use of polyurethane); Fukushima, et al., "Seven-year Follow-up Study After the Replacement of the Esophagus with an Artificial Esophagus in the Dog," 93 Surgery, January, 1983, pp. 70-77 (disclosing a silicone rubber tube with outer Dacron mesh). E. Michelson, et al. review and discuss the many devices for tracheal replacement which have been tested in "Experiments in Tracheal Reconstruction," 41 Jr. Thoracic and Cardiovascular Surgery, June, 1961, pp. 748-58. And Gore-Tex^{R} has been used with ureteral replacement with limited success. S. Varady, et al., "Ureteral Replacement With a New Synthetic Material: Gore-Tex," 128 Jr. of Urology, July, 1982, pp. 171-75.

Several significant problems have been observed when synthetic prosthetic devices are used for replacement or reconstruction, including leakage of the anastomosis between the device and the surrounding tissue, infection, dehiscence of the anastomotic suture line with subsequent extrusion of the prosthesis into the lumen or outside the body, occurrence of fistulous tracts, and migration of the prosthesis. These problems may arise as a result of the type of prosthesis being used and the technique of implantation. For example, leakage of the suture line is generally the result of improper or inadequate suturing and the particular method adopted for interfacing the prosthetic tube with mucosa. Infection may result from leakage at the anastomotic site, or from failure of aseptic technique during implantation of the prosthesis.

Migration and suture line dehiscence, however, are the result of the unique properties of epithelium. Epithelium will continue to grow along a substrate until it contacts other epithelium, and epithelium is innately unable to permanently heal to anything but mucosa. With a non-biodegradable prosthesis, mucosa begins to migrate inside the prosthesis, but the mucosa soon outgrows its blood supply since vessels cannot penetrate certain polymers, e.g., Silastic^{R}, resulting in a slough of mucosa back to the suture line. Subsequently, dehiscence of the

suture line occurs. The mucosa then grows outside the prosthesis, receiving its blood supply from the connective tissue surrounding the prosthesis. This results in extrusion of the prosthesis into the lumen, or the formation of a fistulous tract.

Some of the problems encountered with implantation of synthetic substrate materials have been overcome by the application of collagen to the synthetic substrate. Collagen, a fibrous protein which is the major extracellular structural protein of connective tissue and bone, forms a structural continuum in binding cells together to form tissue. The findings of Y. Shimizu, et al. in "Studies on Copolymers of Collagen and a Synthetic Polymer (First Report)," 5 Biomat., Med., Dev., Art and Org., 1977, pp. 49-66, "Studies on Composites of Collagen and a Synthetic Polymer (Second Report)," 6 Biomat., Med., Dev., Art. and Org., 1978, pp. 375-391, and "Study on Composite of Collagen and Synthetic Polymer," Proceedings of the Second Meeting of ISAO, April 1979, suggested that a copolymer of collagen and a synthetic material is highly effective for use as a prosthetic biomaterial because of the formation and growth of natural connective tissue encouraged by the collagen. A collagenous prosthetic implant with a non-absorbable fabric reinforcement was disclosed in U.S. Patent No. 3,272,204 to Artandi, et al., and collagen-coated silicone rubber useable in surgical treatments was disclosed in U.S. Patent No. 3,955,012 to Okamura, et al.

In prior synthetic substrate-collagen copolymers, the collagen has been shown to encourage epithelial growth across the substrate. The collagen is eventually absorbed by the body, leaving a layer of epithelial tissue positioned on top of the synthetic substrate. Although epithelialization across the substrate does occur in these devices, significant problems have still been encountered. For example, the growing epithelial layer lacks vascularization and growth eventually is restricted. It has also been found that

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epithelialization may not occur completely across the substrate. Further, growth cannot be controlled and excess connective tissue growth may result in stenosis of the tract. Ineffective methods of collagen application have also led to implants which leak.

The problems associated with implantation of synthetic prosthetic devices, as enumerated above, may be overcome by providing a prosthesis which will encourage primary interfacing between the prosthesis and the body's connective tissue which will promote epithelial growth within the lumen of the prosthesis or organ while promoting vascularization and anchoring of connective tissue through the pores of the synthetic material.

SUMMARY OF THE INVENTION

The instant invention presents a unique material for implantation into the tissue or viscera of a body which addresses the difficulties encountered with prior synthetic prosthetic forms. That is, through controlled application of collagen to a synthetic substrate, the invention encourages regulated growth of connective tissue for ultimate incorporation of the device into the surrounding body tissue.

The invention includes a porous synthetic polymeric substrate to which collagen has been applied so that the pores of the substrate become occluded with the collagen, thereby rendering the substrate impermeable to liquid and bacterial invasion. This impermeability is particularly important when the invention is used in urinary tract, respiratory tract, or gastrointestinal tract for reconstruction or replacement.

The synthetic substrate may generally be formed of any tissue-compatible and relatively inert synthetic polymer, such as polyethylene, polyurethane, polypropylene, silicone rubber, or teflon. The pores formed therethrough may have uniform or irregular shape and size. Irregularity of porosity, however, enhances the strength of epithelial bonding to the substrate and also controls

the amount of granulation tissue (initial connective tissue) forming therethrough. A porous synthesized metallic material, such as tantalum, may also be used in the invention. "Synthetic", as used herein, refers to any material, whether metallic, polymeric or of natural origin, which is not otherwise naturally occurring in the desired form and which must be specially fabricated for the purposes of this invention.

Controlled growth of connective tissue into the pores of the substrate is important in assuring optimal incorporation of the invention into the body. That is, the rate at which the collagen is absorbed by the body, and the resulting formation of the connective tissue therefor, affects the rate of epithelialization across the substrate and affects the ultimate rate of incorporation of the device. For example, it has been observed in previous collagen coated implants that if insufficient connective tissue ingrowth occurs through the substrate to the lumen, epithelial growth from the suture line toward the center of the implant stops. This is due to outgrowth of the blood supply in the connective tissue resulting in sloughing of the epithelium back to the suture line. If the epithelium does not grow to cover the ingrowing connective tissues, the connective tissue continues to grow and produces stenosis of the lumen of the viscus. Controlled application of collagen to the substrate ameliorates insufficient ingrowth or excessive ingrowth of connective tissue into the substrate, and regulates optimal growth of connective tissue.

Enhancement and control of connective tissue ingrowth into the substrate, and subsequent epithelialization, may be furthered by the addition of fibroblasts to the collagen as a coating on the substrate. Fibroblasts are fibrillar cells found in abundance in connective tissue, and are the source of collagen in connective tissue. When added to the collagen, fibroblasts interact with the collagen to the substrate, and produce their own collagen, thus promoting the ingrowth of connective tissue

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on the substrate to effect complete incorporation of the implant into the surrounding tissue.

The collagen coating may be cross-linked by any of several means, including irradiation, ultraviolet light, glutaraldehyde, or polyglycerol polyglycidylether (PPE). Cross-linking causes increased density and strength of the collagen and causes the collages to expand slightly within the substrate pores to increase the anchoring of collagen to the substrate. Although cross-linking increases the density and strength of the collagen coating to the substrate pores, cross-linking may not be required or even desirable in some applications.

In addition to the porous synthetic polymer used as a substrate, other synthetic materials may be associated with the substrate which lend elasticity to the implant or prosthesis as needed. That is, in some applications a certain amount of elasticity is desirable. An example would be prosthetic implants for the urinary bladder where expansion and contraction of the implant is required during filling and evacuation. Typical elastic materials which might be used include silicone rubber (Silastic$^{R)}$ or polyurethanes.

Optimum efficacy of the implant may be achieved through association of mucosal epithelium from the surrounding viscus or tissue with the intraluminal surface of the implant. The epithelial lining of many organs is important to the function of that organ. For example, the mucosal lining of the trachea contains cilia which move debris out of the lungs. A graft of mucosal epithelium from the trachea or nasal sinus may be attached to the surface of the implant or prosthesis which will be directed intraluminally. With vascularization through the synthetic substrate and epithelialization, the intraluminal graft may eventually assume its function.

The incorporable material of the present invention may be adaptable to a variety of medical or surgical applications. For example, the incorporable material may be produced in the form of a patch for use in

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repairing or reconstructing holes in a viscus produced by accidental trauma or necessary surgical resection, or for expanding a stenotic lumen. As an example of need for this application, nine percent of infants placed on ventilators develop subglottic stenosis. This often progresses to the point where the glottis is totally occluded. At present, there is no prosthesis available to expand the stenosed glottis and a costal cartilage must be utilized. This procedure usually requires a prolonged period of tracheostomy and a seton in the glottis. The use of a patch that will epithelize rapidly and is totally incorporated may decrease the morbidity in the more severe cases of subglottic stenosis and repair in these patients.

Alternatively, the incorporable material may be formed into a tube for implantation into a trachea, esophagus, or intestine where end-to-end anastomosis is made impossible. Most esophageal resections are performed for cancer of the esophagus or cancer at the cardioesophageal junction. Esophageal resections are also required for some benign lesions and chemical burns. The use of radiotherapy or chemotherapy, either individually or in combination, has not eliminated the continued need for surgical resection of the esophagus for the management of carcinoma of the esophagus. The non-surgical palliative use of the intraluminal esophageal tube to maintain a lumen for the passage of saliva and food has proved to be generally unsatisfactory and inferior to surgical resection as a palliation of esophageal cancer. The intraluminal tube is sometimes used as a last resort, or not used at all.

Both palliative esophageal resection and wide extended resection of carcinoma of the esophagus, with and without the use of chemotherapy and irradiation, provide most patients with a very limited survival time of less than two years from time of diagnosis. Despite the prognosis of limited survival time, many patients are still subjected to a major surgical procedure which may include surgical invasion of both the thorax and abdomen

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for production of a graft. At present, the maximum amount of trachea that can be successfully resected is six to eight centimeters due to tension. A Nevile prosthesis (a medical grade silicone copolymer tube with polyester-covered Dacron sewing rings at either end) is sometimes used, but this does not permit intraluminal connective tissue ingrowth or epithelial ingrowth, and it often extrudes or forms fistulas. These major surgical procedures, in mostly elderly or debilitated patients, may have a high rate of morbidity. The use of an artificial segment of esophagus would allow the surgical procedure to be limited to the thorax and possibly the neck, without the necessity of an abdominal procedure. The elimination of the abdominal procedure would allow most patients immediate restoration of nutrition postoperatively through the gastrointestinal tract. It would also eliminate the problems of post-laparotomy ileus and provide a shorter postoperative hospitalization for these patients who have prognostically a very limited survival time.

In an alternative embodiment, the invention may be used as a prosthetic replacement for an entire organ or organ system. For example, urinary bladder replacement is indicated following cystectomy for bladder cancer. Reported cases of bladder cancer range from 37,000 to 40,000 cases per year, about 25% of which eventually require cystectomy. Additionally, neurogenic disorders and pelvic exenteration (selective evisceration) for cervical or rectal cancer or contracted urinary bladder often require urinary tract diversion or cystectomy. Current methods for post-cystectomy urinary diversion utilize intestinal conduits, usually from ileum or colon. These procedures have a high incidence of complications as well as requiring the use of external appliances, making the patients urologically handicapped.

Many different methods of repair have been proposed and tested over the last few decades, but most methods have failed or have proven insufficient due to infection, inflammation, rejection, extrusion,

obstruction, leakage, or fistula formation. The only methods which have met with even a modicum of success are those in which a portion of the bladder was left intact, thereby providing a source for generation of transitional-cell epithelium.

The present invention, by being capable of incorporation into the surrounding tissues, overcomes the consequences associated with previous prosthetic devices, and provides a functional device in such applications.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a perspective view in partial cut-away illustrating a porous synthetic substrate;2

FIG. 2 is a cross-section view of a substrate illustrating the open-celled arrangement of pores;

FIG. 3 is a cross-section view of a substrate in contact with collagen;

FIG. 4 is a cross-section view of an implanted prosthetic device illustrating ingrowth of connective tissue and a layer of epithelium on the intraluminal and extraluminal surface;

FIG. 5 is a cross-section view of a prosthetic device illustrating host graft epithelium on a intraluminal surface;

FIG. 6 is a perspective view of an embodiment of the invention comprising a prosthetic patch;

FIG. 7 is a perspective view of an embodiment of the invention comprising a hollow tube; and

FIG. 8 is a perspective view in partial cut-away of an embodiment comprising a prosthetic bladder.

DETAILED DESCRIPTION OF THE ILLUSTRATED EMBODIMENTS

The invention generally includes a porous substrate material which is contacted with either collagen or a mixture of collagen and fibroblasts. A layer of autogenous graft or tissue-cultured mucosal epithelium may be applied to one surface of the material, the intra-

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luminal surface, to promote growth of host mucosal epithelium. The prosthetic material of the invention may take any form, including an implantable patch, prosthetic tube, or prosthetic organ. The basic material and prosthetic forms are discussed more fully below.

The Substrate

The substrate of the material may be any porous synthetic material which is tissue-compatible, including metallic materials and organic polymers. Organic polymers which are inert in the body are preferable, such as polyethylene or polyurethane. In a preferred embodiment, the substrate is a polyethylene material which has formed therethrough a substantial number of irregularly shaped, open-celled pores. An example of such material is Medpor manufactured by Porex Medical Co. (Fairburn, Ga.). As illustrated in FIG. 1, the pores 22 in synthetic material 20 of this type are pores which are irregular in shape and size and do not necessarily define a straight path. The diameter of the pores or the pore opening width, as generally measured by the diameter of a sufficiently small object being able to pass therethrough, can vary from a fraction of a micron to a few hundred microns. For the purposes of this invention, a pore size of between about 100 microns and 360 microns is preferred.

The thickness of the substrate affects the rate and amount of connective tissue ingrowth. That is, the thicker the substrate, the greater the depth of the pores through which the connective tissue must grow. Therefore, the thickness of the substrate may vary from between about 0.25 millimeters (mm) to about 1.50 centimeters (cm).

When a thin sheet of this porous synthetic polymer is viewed in cross-section, as illustrated in FIG. 2, it can be seen that the pores 24 follow a tortuous and irregular path. A substantial number of the pores are open-celled 25, meaning that there is an opening to the pore at both surfaces of the substrate. There may, however, be a number of pores which are close-celled 26;

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that is, having an opening only to one surface only. The irregular porosity in the substrate provides a stronger adherence of collagen or collagen and fibroblasts to the substrate. Additionally, the ingrowth of connective tissue through the irregularly shaped pores provides greater strength and adherence of the tissue to the substrate leading to assured incorporation of the prosthesis into the living tissue.

The Collagen

The substrate is contacted with collagen in any manner which allows adherence of the collagen to the substrate and which causes the collagen to fill the pores sufficiently to produce a liquid-impermeable seal. As illustrated in FIG. 3, the collagen 28 substantially fills the pores of the substrate 30 and defines a thin layer of collagen 32 on one surface of the substrate.

The prosthetic material of the present invention allows controlled ingrowth of epithelium by controlling the ingrowth of vascularized connective tissue. This is done by varying the thickness and/or density (i.e the concentration of collagen in solution) of the collagen applied to the substrate, and by varying the thickness of the substrate. Thus, where the collagen or collagen-fibroblast coating on the substrate is thinner, more rapid connective tissue ingrowth occurs. Conversely, when the collagen coating is thicker and/or more dense, less connective tissue ingrowth occurs. The thickness of the collagen may be from about 0.025 mm to about 1.00 mm.

The more rapid the connective tissue ingrowth, the more rapid the epithelial growth. Therefore, more connective tissue ingrowth and epithelial growth at the point of suturing may be encouraged by a thinner and less dense layer of collagen or a thinner substrate at that point. Conversely, stenosis of the lumen produced by excess connective tissue growth in the inner portion may be avoided by a thicker and more dense layer of collagen or a thicker substrate. This will allow the

epithelization to continue. The rate of absorption of collagen, and subsequent ingrowth of connective tissue, may also be controlled by varying the depth to which collagen or collagen and fibroblasts are applied in the pores of the substrate. Collagen absorption may also be controlled by cross-linking of collagen, as described below.

FIG. 4 illustrates how collagen 36 is replaced by connective tissue 38 which grows into pores 40 of the substrate 42. The vascularization 44 of the connective tissue retains the viability of the tissue so that epithelium 46 may grow.

Any type of non-antigenic collagen may be used to coat the substrate. Preferably, atelocollagen (Koken Tokyo, Japan) is used. Atelocollagen is an enzyme-solubilized collagen lacking in the strongest antigenic determinants and, as a result, has little antigenic activity to the body. Atelocollagen is biodegradable, shows significantly little inflammatory response, and is particularly suitable for sealing the pores of the substrate due to its viscosity.

Atelocollagen purchased from Koken Co., Ltd., (5 -18, Shimoochiai 3-chome, Shinjuku-Ku, Tokyo, 161 Japan) is kept in its dry form in a refrigerator at approximately 40° F. The dry atelocollagen is solubilized in 0.01M CH_3COOH (glacial acetic acid) in a suitable size (usually 20 ml) glass vial with a screw cap.

As previously noted the rate of collagen absorption may be regulated by varying the density of collagen which is applied to the substrate. Density, as used in this disclosure, refers to the concentration of collagen molecules in solution. The concentration of collagen may vary from one to five percent.

By way of example, a 5% solution of collagen is prepared by adding one gram of solubilized collagen to nineteen milliliters (ml) of 0.10M CH_3COOH. The solution is maintained for about 24 hours to completely dissolve the collagen. The solution is stirred two to three times

during this period. The collagen is then stored in the refrigerator. Once the collagen is dissolved, the solution may be used for up to four months after it is prepared if no signs of contamination are present. The concentration of collagen in the solution is determined according to the desired use of the device. Thus, if fast dissolution of the collagen is necessary, a lower percentage of collagen is used. If slower connective tissue ingrowth is required, a higher collagen percentage is used.

One method of contacting atelocollagen with the substrate may be obtained by the method set forth in the following example:

Example A:

A piece of Medpor (Porex, Inc., Fairburn, Ga.) having a pore size of approximately 100 to 300 micrometers was cut with scissors to an approximate patch size of two centimeters (cm) by four cm. The patch was weighed, measuring 14 gm. An equal amount of prepared 5% solubilized collagen, 14 gm., was weighed out. The collagen was spread over the Medpor with a spatula to coat the upper surface of the Medpor. The coated Medpor was immediately immersed in 100 ml of 5% NaCl solution for one hour. The collagen became cloudy and began to produce random, non-parallel fibrils of collagen. Alternatively, the coated piece of Medpor may be immersed in 100 ml of phosphate buffered saline (PBS) immediately after coating the piece of Medpor. This procedure produces native or parallel placed fibrils of collagen.

Adherence of Collagen to the Substrate

The contacting of collagen to a porous substrate of irregular porosity increases the anchoring of the collagen to the substrate due to the collagen seeping into the labyrinthine cavities of the pores. As a result of this mechanical anchoring, additional bonding between the

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collagen and substrate is not necessary. Further, when atelocollagen is mixed with fibroblasts in culture medium, as set forth in Example C, below, the fibroblast culture medium causes the atelocollagen, a normally gelatinous substance, to become more liquid in consistency. The contacting of atelocollagen and fibroblasts to the substrate, in that liquified state, increases the ability of the atelocollagen to seep into the irregular pores of the substrate so that further bonding is not required.

Cross-Linking of Collagen

Two and one-half percent to five percent atelocollagen is normally very soluble and will not maintain itself in a fluid environment. In order to decrease the solubility of atelocollagen and have it persist longer before being absorbed by the body, it may be cross-linked. Cross-linking increases the density of the collagen by inducing linking between the collagen polymer chains. The greater the degree of cross-linking the more dense and less biodegradable is the collagen. Thus, it can be appreciated that the rate of absorption of collagen is dependent upon the concentration of collagen in the coating, the amount of cross-linking between these collagen chains, and the degree of porosity or void volume of the pores in the substrate.

Cross-linking is a technique well known in the art and can be accomplished by many methods, including irradiation, ultraviolet light, or chemicals such as glutaraldehyde.

Methods for inducing cross-linking by use of glutaraldehyde are discussed in D. T. Cheung and M. E. Nimni, "Mechanism of Crosslinking of Proteins and Glutaraldehyde I: Reaction with Model Compounds," 10 Connect. Tiss. Res. 187-99 (1982) and D. T. Cheung and M. E. Nimni, "Mechanism of Crosslinking of Proteins by Glutaraldehyde II: Reaction with Monomeric and Polymeric Collagen," 10 Connect. Tiss. Res., 201-16 (1982), the contents of which are incorporated herein by reference.

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Methods for inducing crosslinking by use of irradiation are discussed in R. J. Davidson and D. R. Cooper, "The Effect of Ultraviolet Irradiation on Acid-Soluble Collagen," 105 Biochem. J., 965-69 (1967), D. R. Cooper and R. J. Davidson, "The Effect of Ultraviolet Irradiation on Soluble Collagen," 97 Biochem J., 139-47 (1965), and T. Miyata, T. Sonde, A. L. Rubin, and K. H. Stenzel, "Effects of Ultraviolet Irradiation on Native and Telopeptide-poor Collagen," 229 Biochim Biophys. Acta, 672-680 (1971), the contents of each of which is incorporated herein by reference.

An example of induced cross-linking follows:

Example B:

A substrate measuring 2 cm x 4 cm was coated with collagen (as described in Example A, above), and precipitated by immersing the coated substrate patch in 5% NaCl solution. One hundred milliliters of glutaraldehyde crosslinking solution were prepared by adding together 50 ml of MeOH and 50 ml of H_2O. The solution was maintained in a 150 ml Erlenmeyer flask on a magnetic stir plate. The pH of the solution was adjusted to 11.5 by adding 0.5 ml of 1 N NaOH. Forty milliliters of 25% glutaraldehyde were added to the solution, and the pH was adjusted to 12 by the addition of 0.1 ml of 1N NaOH. The solution was stirred slowly for one hour on the plate.

Immediately following precipitation in the collagen with NaCl, the patch was immediately placed into glutaraldehyde solution in a capped vial. The patch was completely submerged in the solution.

The vial was placed in a shaking water bath at 25-26°C. for two hours to produce a high degree of cross-linking. (Lesser time is required if faster dissolution of the collagen is required.)

After two hours had lapsed, the patch was removed from the glutaraldehyde solution and placed in 100 ml. of phosphate buffered solution containing sodium azide

(NaN_3). (Phosphate buffered solution is prepared by adding 0.272 gm/l potassium phosphate (KH_2PO_4), 1.136 gm/l sodium phosphate (Na_2HPO_4), and 8.474 gm/l sodium chloride (NaCl) to 250 ml distilled water, then adding a quantity of distilled water sufficient to make 1000 ml of solution. To that is added 1 gm/l of sodium azide (NaN_3).)

The patch in PBS and sodium azide was kept in a shaking water bath for 5 to 7 days at 25-26°C. The solution was changed twice a day for the first 3 days, and at least once a day thereafter. This procedure of changing solutions was performed to remove any unreacted glutaraldehyde which might have been left in the collagen.

At the end of this period of time, the patch was kept in saline solution with sodium azide and kept in the refrigerator until 24 hours prior to surgical implant.

Approximately twenty-four hours before implant, the patch was removed from the sodium azide-saline bath. It was then placed in a sterile container with sterile saline. The saline was changed, using sterile techniques, five to six times in the first 12 hours. The final change was into sterile saline containing 1 gm/1000 ml cefadyl solution. The patch was stored in the refrigerator during this time.

At this point, devices prepared by the foregoing procedure are considered ready for implantation, and are to be handled using sterile techniques.

The Fibroblasts

The substrate may be contacted with either atelocollagen alone, as described above, or an admixture of atelocollagen and fibroblasts may be applied to the substrate. Fibroblasts are present in all types of fibrillar tissues and membranes, such as areolar, dense fibrous, and elastic tissue, and are the source of collagen fibrils. Fibroblasts which have been tissue-cultured are added to atelocollagen and are then contacted with the porous substrate. The fibroblasts produce their

own collagen and cause the atelocollagen to shrink and become more dense. Because this shrinking and increased density causes the collagen-fibroblast coating to adhere more strongly to the pores of the substrate, cross-linking is not required. Additionally, cross-linking may damage or kill the living fibroblasts. The living cellular content formed by the fibroblasts within the substrate enhances the ingrowth of connective tissue. This living membrane within the substrate should also transfer nutrients across the substrate more rapidly than cross-linked atelocollagen. The transfer of nutrients is important to the growth of connective tissue and therefore ensures better survival of free mucosal or tissue-cultured mucosal grafts.

One method of contacting a porous substrate with a collagen and fibroblast mixture may be illustrated by the following example.

Example C:

Fibroblast Culturing

Canine buccal mucosa is harvested, washed with 1 liter (l) phosphate buffered solution (PBS), and cut with a knife into small explants of approximately 2 cm by 2 cm. These explants are placed on the upper seam of a 25 cubic cm flask and are fed 9 ml of Dulbecco's modified Eagle medium (DMEM) with 10% Fetal Bovine serum (FBS).

Dulbecco's modified Eagle medium is a tissue culture medium purchased from Gibco Labs, Grand Island, N.Y. The medium is prepared by mixing together 1000 ml of distilled, deionized water, 10 grams (g) DMEM powder (#430-1600, Gibco Catalog), 3.7 g sodium bicarbonate, 0.3 g l-glutamine, 4.76 g N-2 hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES), 2.5 mg/10.5 ml Amphotericin-B, and 10 ml Penicillin-Streptomycin. The pH of the mixture is raised to 7.3 with 1M NaOH. The mixture is filtered using sterile techniques. Then 100 ml of fetal bovine serum (FBS), purchased from Hyclone Labs, Logan,

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Utah, is added. Fetal bovine serum is blood serum which is extracted from fetal calf blood, and it provides a nutrient medium for cell growth.

These flasks are incubated at 37° centigrade (C) at a slight angle so that the medium comes only to the seam of the flask. Fibroblasts begin to grow out of the explants and to migrate down into the flask. After three weeks, when the fibroblast cells are growing well, the cells are transferred to culture dishes and allowed to proliferate in DME culture medium for seven days.

Fibroblast Coating

Patches of Medpor^{R} porous synthetic substrate measuring 2 cm by 3 cm are coated with 1 ml of 1.25% atelocollagen applied by pipette. The coated patches are allowed to stand for 16 to 24 hours at 37°C to seal the pores of the substrate. Thereafter, 1 ml of 1.25% atelocollagen containing approximately 4 x 10^{6} fibroblasts is pipetted on each patch. The coated patches are then placed in 5 ml DME with 10% FBS. As the fibroblasts proliferate, they begin collagen production of their own. After two weeks, the patches are tested for leakage. This is done by placing the patch on a ring above the medium dish. One-half milliliter of DMEM is pipetted onto the patch, and the patch is observed for five minutes to detect any filtering of DMEM through the patch. If any leakage is observed, another 1 ml of 1.25% collagen and fibroblasts is added to each patch as before. Testing for leakage is done every two weeks until the patches are sealed.

Example D:

Autogenous Graft Epithelium

Many types of cells of epidermal and endodermal origin, when placed on a membrane-like extracellular matrix, such as collagen, are enhanced by the culture environment such that cell proliferation,

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cytodifferentiation, and gene expression are promoted. Such growth and differentiated function has been demonstrated using human hepatocarcinoma and Ewing's sarcoma, bovine vascular endothelium from aorta, pulmonary artery, umbilical vein, vena cava, bovine capillary endothelium from adrenal cortex, brain cortex, corpus luteum, and human upper respiratory epithelium from nasal polyps. Such cells were previously resistant to successful attempts at culture. However, when placed in serum-supplemented culture on the collagen, they attach firmly, migrate, and proliferate to form a flattened, non-overlapping, closely apposed epithelial layer. Functional characteristics, such as mucous secretion, ciliary movement, expression of tissue specific antigens, and other similar characteristics are demonstrated. Generally such cultures resemble the in vivo counterparts in each of the ways thus far measured.

It is clear that the use of autogenous grafts of epithelial tissue, such as buccal mucosal epithelium, will enable the prosthetic device to become incorporated more readily into the host tissue by allowing the prosthesis to become physiologically functional within a shorter period of time. As used herein, "autogenous" refers to donor tissue taken from the host viscus or surrounding tissue. The tissue may be taken from the same individual upon whom the surgical procedure is being performed. FIG. 5 illustrates the placement of graft epithelium 50 on a substrate 52 which has been coated with collagen 54 and fibroblasts 56.

Autogenous graft epithelium may be applied to the collagen- or collagen/fibroblast-covered substrate in either a direct manner, or through in vitro culturing. These two procedures are illustrated by Example E and Example F, respectively.

Example D

A collagen-coated substrate, readied for implantation, is placed within a surgical bowl. The sub-

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strate is then pre-clotted with blood taken from the patient. A strip of buccal or respiratory mucosa 1/2 cm wide is harvested from the patient. The strip is sutured onto the lumen surface of the substrate with absorbable suture. The lumen surface may be coated with a synthetic biodegradable material such as fibrin glue to protect the graft. The substrate is sutured into place in the area to be repaired. During the implant procedure, the device is kept moist with Dulbecco's tissue media.

Example F:

Patches of Medpor (Porex, Inc., Fairburn, Ga.) are soaked in saline with 0.1% cefadyl for approximately 48 hours to remove any residual ethanol or sodium azide in which they are stored. They are then pre-equilibrated in Dulbecco's modified Eagle tissue culture medium for approximately 24 hours.

Surgically excised canine buccal or urothelial tissue is washed in phosphate buffer saline (PBS) and the top portion of the dermis and the epithelium are removed using a dermatome (slicer) set to cut at 500 um. The lower dermis is discarded. The epithelium-dermis is then cut into small explants approximately 1 to 2 mm^2. These explants are placed on the collagen-coated side of the patch or that which will become the inter-luminal surface (approximately 5 explants per cm^2). The patches are then placed in 60 x 15 mm tissue culture dishes and are fed 5 ml of tissue culture medium [Keratinocyte Growth Medium (KGM), Clonetics Corp., San Diego, CA]. For urothelium, KGM is mixed with Dulbecco's modified Eagle medium (DMEM) in a ratio of 90 KGM:10% DME containing 10% FBS. The patches are cultured at 37° for 14 days. At that time, the patches are epithelized sufficiently to permit implantation.

Once the prosthesis has been implanted, the collagen or collagen-fibroblast layer prevents bacteria or contamination from penetrating into the substrate while

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allowing connective tissue (granulation tissue) to grow into the substrate from the surrounding tissue. As illustrated in FIG. 4, by the time the collagen, collagen/fibroblast layer has been biodegraded by the invading connective tissue, the pores 40 are filled with a bacteriocidal layer of granulation tissue 38 (immature connective tissue). This granulation tissue provides the blood supply necessary for the epithelium to survive and for the epithelium to ingrow from the suture site. Collagen fibroblasts also allow the osmotic transport of nutrients from the surrounding tissue to keep the epithelium viable and growing.

Prosthetic Forms

The basic material of the prosthesis may be formed into any number of devices for use in reconstruction of areas of damage, or for replacement of entire viscera. In one embodiment, the material may be formed into a patch, or single layer of collagen-covered substrate, as illustrated in FIG. 6. This particular embodiment may be used to reconstruct areas of damage in tissues or viscera where end-to-end anastomosis of the tissue or viscera is impossible. The patch may be made in any shape or size as dictated by the area to be reconstructed. FIG. 6 illustrates, by way of example, a rounded piece of porous substrate 60 which has been coated, in part, by a film of collagen 62, and is thus ready for implantation.

In an alternative embodiment, a tube 64, as illustrated by FIG. 7, can be formed from the porous synthetic substrate and contacted with collagen 66 or collagen and fibroblasts. An additional layer of autogenous graft epithelium may be added. The tube thus formed can be used for reconstructing the trachea, esophagus, or varying lengths of the intestinal tract where end-to-end anastomosis is made impossible by resection of too much tissue.

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In another alternative embodiment, the basic material may be formed into a prosthetic organ, such as a urinary bladder, for replacement of the natural organ. FIG. 8 illustrates a urinary bladder 68, shown in partial cut-away, formed from porous synthetic substrate 70. In this embodiment, it may be desirable to attach an autogenous layer of, for example, bladder mucosal tissue 76 to the intraluminal surface, as shown in FIG. 8. The ureters 72 and urethra 74 of the patient have been sutured to the prosthesis.

Examples illustrating procedural techniques for implantation of the various embodiments of the device, and results of incorporation of the device are given below.

EXAMPLE I:

Trachea or glottis patch implants

The trachea or glottis of a patient is longitudinally incised. A patch is selected which is the same longitudinal length as the defect. The width of the defect is determined by the size of the area to be patched. Patches measuring 2 by 3 cm having coatings of 5% collagen, 2 1/2% collagen, 2 1/2% collagen/fibroblast, and 2 1/2% collagen fibroblasts with buccal mucosa tissue culture have been implanted. The mucosa is dissected from the cartilage creating a 2 mm flange of tissue.

The mucosal flange is sutured to the luminal aspect of the patch with a continuous preplaced absorbable suture. After the mucosal suture is tied, the cartilage is placed in the groove or under the flange and fixed with interrupted non-absorbable sutures and pledgets.

There was no evidence of air loss or infection. The initial patches of 5% collagen dislodged and did not show ingrowth. Patches of 2 1/2% collagen demonstrated ingrowth through the edges of the patch but no epithelial growth from the edges. Patches of 2 1/2% collagen/fibroblast demonstrated tissue growth in the center of the patch. Patches of 2 1/2% collagen fibroblast buccal

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mucosa demonstrated tissue growth in the center of the patch. Patches of 2 1/2% collagen fibroblast buccal mucosa demonstrated tissue ingrowth with some evidence of tissue cultured mucosal survival. The groove shaped patch demonstrated that mechanical fixation can occur without connective tissue ingrowth.

Tracheal tube implants in dogs up to 7 cm long with 5% collagen coated tubes demonstrated complete connective tissue ingrowth through the substrate and an intraluminal connective tissue layer. Stenosis of the center of some substrates occurred because of excess connective tissue growth.

Epithelial ingrowth from the sutures line occurred up to 2 cm from each end. One tube, with a free buccal mucosal graft demonstrated complete epithelization of a 7 cm long tube.

EXAMPLE II:

Five percent collagen coated tubes up to 5 cm long were implanted in an isolated bowel segment in dogs. The bowel segment was isolated. The mucosa-submucosa was dissected from the sero-muscular layer. The mucosa was sutured inside the lumen of the implant with pre-placed continuous absorbable suture. The sero-muscular layer was sutured on the outside of the implant with continuous suture of non-absorbable material. One end of the isolated bowel was closed; the other end was brought to the outside as a colostomy. The implant was wrapped with omentum and the omentum was sutured in place about the tube. There was no leak or infection. All tubes demonstrated a luminal layer of connective tissue by 4 weeks post-operatively. At sixty days, there was evidence, in some tubes, of mucosal migration from the suture line toward the center of the device for a distance of .6 mm from each end. (Normal bowel will only show .8 mm of epithelial growth.) There was production of mucus and fluid and high bacterial count in the isolated bowel.

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EXAMPLE III:

Patches measuring 2 by 4 cm in size with a coating of 5% collagen, 2 1/2% collagen, 2 1/2% collagen/fibroblasts with autologous uroepithelium or buccal epithelium tissue cultured onto the collagen were implanted in the urinary bladder of dogs. The urinary bladder was exposed. A deffect the same size as the patch was created in the bladder. The mucosa-submucosa was dissected from the sero-muscular layer creating a 2 mm flange. The mucosal layer was sutured to the cultured (lumen) surface of the patch with a continuous suture of absorbable material. The sero-muscular layer was sutured to the outside of the patch with absorbable continuous suture. The patch was covered with omentum and sutured in place. There was no evidence of infection or urine leakage. Most patches demonstrated connective tissue ingrowth around the edges. The 2 1/2% collagen/fibroblast/cultured patch demonstrated connective tissue ingrowth in the center of the patch and some evidence of tissue cultured cell survival.

CLAIMS

What is claimed:

1. A prosthetic device suitable for implantation in living tissue of a body, said prosthetic device comprising:
a thin synthetic substrate having irregularly shaped pores and defining opposing surfaces; and
collagenous material in contact with at least a portion of at least one said surface of said thin synthetic substrate.

2. The prosthetic device of Claim 1, further including fibroblasts in contact with said collagenous material.

3. The prosthetic device of Claim 2 wherein said collagen is atelocollagen.

4. The prosthetic device of Claim 1 wherein said collagenous material is a solution wherein the concentration of collagen varies from between 1.5% collagen and 5.0% collagen.

5. The prosthetic device of Claim 1 wherein a thickness of said collagenous material varies from 0.025 mm to 1.0 mm.

6. The prosthetic device of Claim 1 wherein said collagenous material contacts said thin synthetic substrate with sufficient thickness to substantially occlude said pores.

7. The prosthetic device of Claim 1 wherein said thin synthetic substrate varies in thickness from 0.25 millimeters to 1.5 centimeters.

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8. The prosthetic device of Claim 1 wherein said thin synthetic substrate has a first intraluminal surface and wherein autogenous epithelial tissue is adhered on said first intraluminal surface over said collagenous material.

9. A method of producing a prosthetic device suitable for implantation into living tissue of a body, said method comprising:

providing a synthetic substrate having opposing surfaces and irregularly shaped pores; and

applying a collagenous material to said synthetic substrate on at least one said surface to seal said pores with said collagenous material.

10. The method of Claim 9, further comprising treating said synthetic substrate to effect cross-linking of the collagen in said collagenous material and subsequently soaking said synthetic substrate to remove residual substances.

11. The method of Claim 9, further comprising contacting said synthetic substrate with cultured living fibroblasts subsequent to applying said collagenous material.

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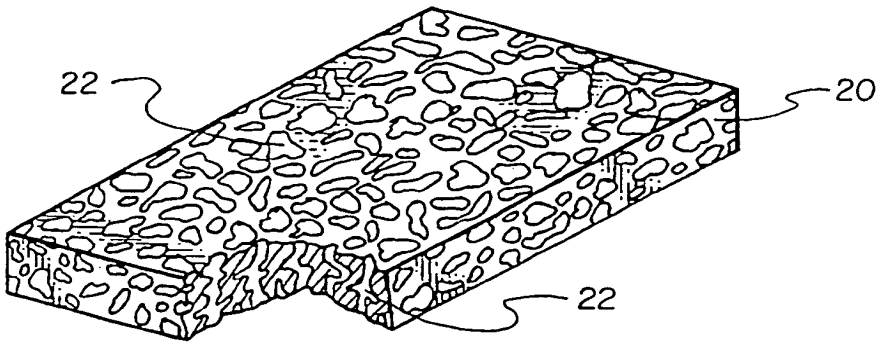

Fig. 1

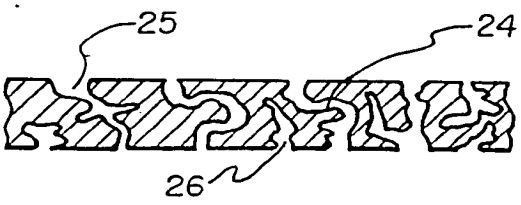

Fig. 2

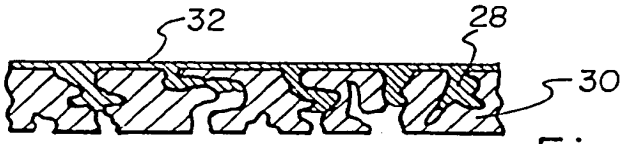

Fig. 3

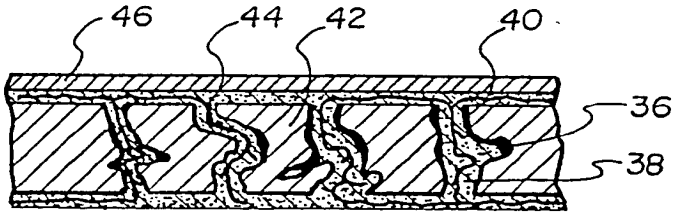

Fig. 4

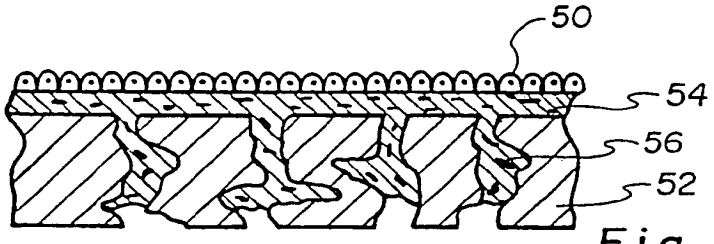

Fig. 5

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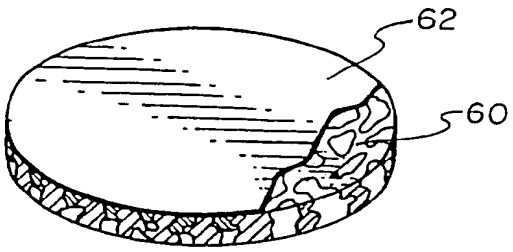

Fig. 6

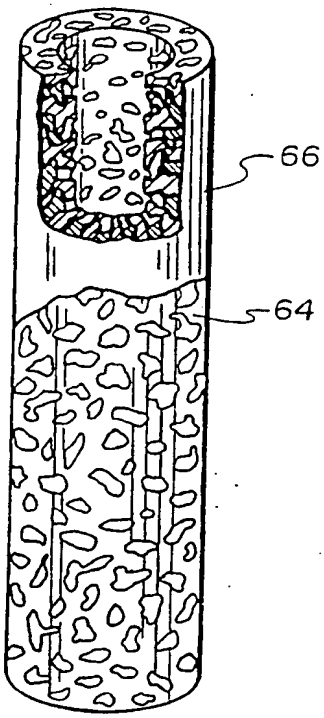

Fig. 7

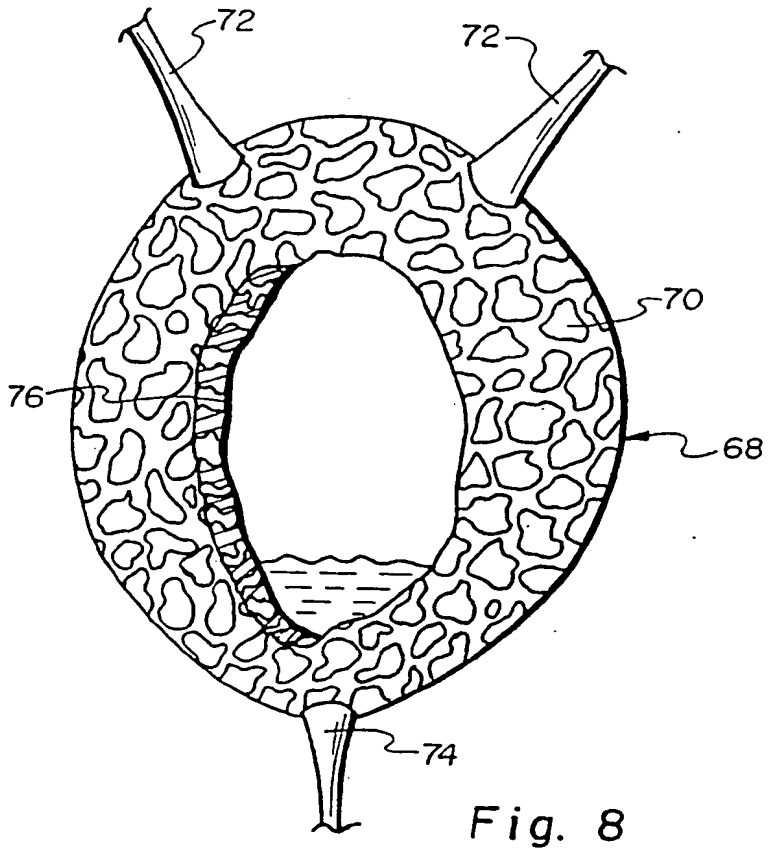

Fig. 8

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INTERNATIONAL SEARCH REPORT

International Application No. PCT/US90/07233

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5): A61F 2/04
US CL.: 623/12

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
US	623/1,12,15,66 600/36 435/240-242

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
Y	US, A, 4,842,575 (HOFFMAN, JR. et al.) 27 June 1989 See column 4, lines 30-31; column 2, lines 11-16.	1,2,4-11
Y	US, A, 4,539,716 (BELL) 10 September 1985 See column 1, line 50.	1,2,4-11
Y	US, A, 4,642,117 (NGUYEN) 10 February 1987 See column 1, lines 12-30.	3

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
07 MARCH 1991	04 APR 1991
International Searching Authority	**Signature of Authorized Officer**
ISA/US	DAVID J. ISABELLA

Form PCT/ISA/210 (second sheet) (Rev.11-87)

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